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Review on the developmental approach of Ecdysis derived scaffold in organ regeneration particularly hepatic cell regeneration: An idea based Hypothesis

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ABSTRACT:

The developing field of tissue engineering aims to regenerate damaged tissues by combining cells with highly porous ecdysis derived scaffold biomaterials which might be act as templates for organ regeneration and for the growth of new tissues. The organs particularly, liver which shows a unique ability for regeneration with the prospective of full restoration of cell mass. However, it is seen that number of patients increases regarding hepatic transplant increases due to hepatic failure caused by sickness, hereditary complications or antagonistic medication responses and there are not enough organ donors. So, this idea can help to encourage the further research in the the functional requirements, and types, of materials used in developing state of the art of ecdysis derived scaffolds and its applications in tissue engineering. Literature survey and analysis reflected that this study might be helpful to understand the utility and developing a scaffold, derived from waste exoskeleton material known as ecdysis which as an alternative functional material with sufficient mechanical strength for organ regenerative applications.

KEYWORDS: *Ecdysis, Scaffold, Organ regeneration, Hepatic, Tissue engineering*

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INTRODUCTION OF ECDYSIS

Ecdysis, defined as when the animal sheds the old exoskeleton while absorbing huge amounts of water to stretch the new exoskeleton[1, 2]. During subsequent metecdysis, the synthesis and calcification of the new exoskeleton is accomplished and claw muscles are re-established [3, 4]. For example in the case of insects where Insect cuticles form an exoskeleton that exhibits only a limited capacity to keep pace with body growth due to its more or less rigid structure because of presence of chitin and sclerotized proteins. Insects are thus periodically forced to switch their old cuticle with a new and looser one while molting to allow the further growth and development. This process i.e. Ecdysis is originated by apolysis, resulted in division of the epidermal cells from the old cuticle by molting fluid secretion and ecdysial membrane formation. This fluid comprises proteases and chitinases, enzymes that digest the main elements of the old endocuticle[5]. Before ecdysis, the molting fluid, which has gathered in the apolysial space, becomes reabsorbed that allows the recycling of old cuticle constituents. Then, formation of the new cuticle starts after the ecdysial space opens as a result of the secretion of cuticle proteins and chitin fibers through the apical membranes of epidermal cells. Initially, coverings of cuticullin plays role in the formation of epicuticle, followed by an unsclerotized, chitinous cuticle which is referred as procuticle. Later, development of the epicuticle closures the epidermis and defends it against the digestive enzymes of the molting fluid. Before sclerotization is accomplished, the insects enlarge their fresh cuticle and shack their old covering which is now known as called exuvia, by performing distinct motor programs and increasing body pressure[6]. The behaviour before the process of ecdysis are controlled by the action of molting hormones such as eclosion hormone, which is secreted in response to falling ecdysteroid titers and also causes the release of pre-ecdysis triggering hormone and ecdysis-triggering hormone[7,8,9]. Ecdysis process permits broken tissue and missing limbs to be redeveloped or re-juvinate. Along with this, whole regeneration may need a sequence of moults, the stump fetching a little larger with each moult until it is a normal, or near normal, size[10]. The main objective of this paper to review the does the ecdysis based scaffold in organ regeneration (focus on hepatic problems) becomes the developmental approach in the cell based technologies or medical sciences field.

PREVIOUS REPORTS RELATED TO DIFFERENT ASPECTS OF ECDYSIS

Silverman and Podger[11]observed the *In vitro* ex sheathment of some nematode infective larvae and found that *Trichostrongylus colubriformis* shows a relative requirement for pepsin. Although it can be induced to exsheath in a buffer solution (pH 1.7) under 100% CO_2 gas, it undergoes more rapid and complete ecdysis when pepsin is present in the solution and *Haemonchus contortus* infective larvae are indifferent to pepsin. They undergo rapid and complete exsheathment

in a variety of balanced salt solutions provided that the solutions are under a CO_2 gas phase. Although reducing agents were found to be highly effective in reactivating inactive rumen fluid as an exsheathing medium, they had no enhancing effect in the salt solutions when CO_2 gas was present at high concentrations. Berntzen[12] studied the Comparative growth and development of *Trichinella spiralis in vitro* and *in vivo*, with a re-description of the life cycle in which observed that in good cultures, only one molt or ecdysis was observed during growth and differentiation from excysted larvae to gravid adults and also found that a reduced oxygen level, the presence of carbon dioxide, and low levels of reducing agent provided the stimulus for normal sheath formation *in vitro*. These conditions had to be maintained constant throughout the culture period. Variation of one of these factors during culture induced multiple sheathing. Davey and Kan [13] observed the last ecdysis of *Phocanema* in in vitro culture in which they observed that Ecdysis is accompanied by the synthesis in the excretory gland of the enzyme leucine amino peptidase and its release via the excretory duct into the space between the two cuticles and the excretory system of *Phocanema* is described, and histochemical evidence presented for protein synthesis in the gland during ecdysis. Akai and Sato[14] studied the ultra-structural study of the haemopoietic organs of the silkworm, *Bombyx mori* in which they observed that haemopoietic organs increased in size and number during larval development and the size of the organs increases only during the early stages of each of the instars, and the number of the organs increases only during each apolysis to ecdysis. According to Davey and Goh[15] that Ecdysis in a parasitic nematode which is direct evidence for an ecdysial factor from the head and indicated that incubating intact *Phocanema decipiens* . Scott-Fordsmand and Depledge[16] studied the changes in the tissue concentrations and contents of calcium, copper and zinc in the shore crab *Carcinus maenas* (L.) during the moult cycle and following copper exposure during ecdysis and also observed that during early post moult calcium stored in the mid gut gland was used in combination with calcium absorbed from the surrounding seawater for a rapid calcification of the new exoskeleton.

According to the study conducted by Park *et al* [17] observed the deletion of the ecdysis-triggering hormone gene pointers to lethal ecdysis deficiency and found that at the end of each developmental stage, insects perform a stereotypic behavioural sequence leading to ecdysis of the old cuticle. They also revealed that ecdysis-triggering hormone (ETH) is sufficient to trigger this sequence; it has remained unclear whether it is required. These types of findings inaugurate obligatory parts for ETH in beginning and regulation of the ecdysis sequence. This type of work was also conducted by Dai and Adams[18] in which they studied that ETH signaling in the yellow fever mosquito *Aedes aegypti* and suggested the future aspects directed toward hormone-based interference approaches for controller of mosquitoes as human disease vectors. In addition to this,

Kim *et al* [19]studied the function of Corazonin in regulation of insect ecdysis and found that Corazonin, a highly conserved neuropeptide hormone of extensive occurrence in insects, is added factor essential in the regulation of ecdysis and circulates in the hemolymph. Also, Mykles[20]studied the Interactions between Limb Regeneration and Molting in Decapod Crustaceans and showed that Molting and regeneration of lost appendages are tightly-coupled, hormonally-regulated processes in decapod crustaceans using the process of ecdysis. Kumar *et al*[21]studied the Molecular cloning and structural characterization of Ecdysis Triggering Hormone from *Choristoneura fumiferana* in which they observed Ecdysis is initiated by the direct action of ETH on the CNS (central nervous system). Lenaerts *et al*[22] studied the ecdysis triggering hormone system is essential for successful moulting of a major hemimetabolous pest insect, *Schistocerca gregaria* in which they revealed that in Holometabola, ETH is the key factor in this cascade and Silencing of *SchgrETH* and *SchgrETHR* resulted in lethality at the expected time of ecdysis, thereby showing their crucial role in moulting.

IMPLEMENTATION OF THE CONCEPT OF SCAFFODING DERIVED FROM ECDYSIS

Tissue engineering needs a porous, biodegradable scaffold to reproduce the natural extracellular matrix (ECM), which assists to establish cells spatially, deliver them with environmental signals and undeviating site-specific cellular regulation[23].Pore size and number, also surface area is extensively predictable as significant factors for scaffolds used in tissue engineering. Along with this, many more architectural characters via pore shape and pore wall morphology of the scaffold material have also been recommended as chief factors for cell seeding, migration, growth, and new tissue development in three dimensions[24,25]. The implementation and feasible biomaterial used for scaffold which provides encouragement in the field in tissue engineering. The scaffolds used have low biomechanical stiffness and rapid biodegradation. Furthermore, for tissue engineering, some studies have been reported which demonstrated that high mechanical strength is important for biodegradable polymer scaffolds[26, 27, 28, 29]. Chaudhari *et al* [30] also reviewed about the future prospects for Scaffolding methods and Biomaterials in Skin Tissue Engineering. Similar findings were reported by Lin *et al*[31] in which they derived scaffold from fish scales, for corneal regenerative applications and provided the evidences for the feasibility of the scaffold as a template for corneal cells growth and migration, and thus the this scaffold can be developed as a promising material for tissue-engineering of cornea. Horst *et al* [32]studied the Scaffold Characteristics for Functional Hollow Organ Regeneration in which observed that many medical conditions require surgical reconstruction of hollow organs. Parekh *et al* [33] investigate the biocompatibility of fish scale-derived scaffolds (FSS) with primary human corneal endothelial cells (HCEnCs). Tissue engineering of organs and tissues is an encouraging new technique deprived of harvest site indisposition. An epitome

biomaterial should be biocompatible, support tissue development and provide suitable structural support. It should degrade regularly and deliver a situation permitting for cell-cell interaction, adhesion, proliferation, migration, and differentiation. Though tissue development is possible, functionality has never been verified. This might be due to lack of proper innervation and vascularisation is delaying contractility and normal function.

NEED AND FUTURE ASPECTS OF ECDYSIS AND SCAFFOLDING ON MANY HEALTH PROBLEMS PARTICULARLY FOCUSSED ON HEPATIC BASED PROBLEMS

Consistently, number of patients requiring a hepatic transplant increases due to hepatic failure caused by sickness, hereditary complications or antagonistic medication responses. Presently, there are numerous individuals holding up to have a liver transplant. However, there are not enough organ donors. So, Cell-based treatments have long held guarantee as an option in contrast to organ transplantation[34]. The liver also shows a unique ability for regeneration, with the prospective for full restoration of liver mass and function even after enormous damage in which less than one-third of the cells remain uninjured[35]. Same type of work has been conducted by Yang *et al* [36] showing liver organogenesis (Figure 1). But still, the potential for liver regeneration is often difficult to predict clinically and criteria for identifying patients that may resolve liver failure complications due to regenerative responses remain poorly defined. There are thousands of surgical processes which are performed to replace or repair tissue that has been damaged due to disease or trauma. The emerging field of tissue engineering purposes to regenerate broken tissues by combining cells from the body with highly porous scaffold biomaterials, which act as templates for tissue regeneration, to guide the development of new tissue.

Many regenerative centres worldwide continue working to provide a reliable source of scaffolds and to find feasible strategies in order to develop bioengineered liver. But, bioengineering poses a number of questions and ambiguities. It is a crucial challenge to select suitable source of organs, standardization of obtained scaffold and seeding them with cells, achieving proper organ revascularization and their storage. Along with this, number of ethical and legal issues which are also part of this field. So by using the cell-scaffold technology constitutes one of the possible alternative ways to treat patients suffering from such diseases. However, In-depth revelation on internal mechanisms will lead the development of this research field.

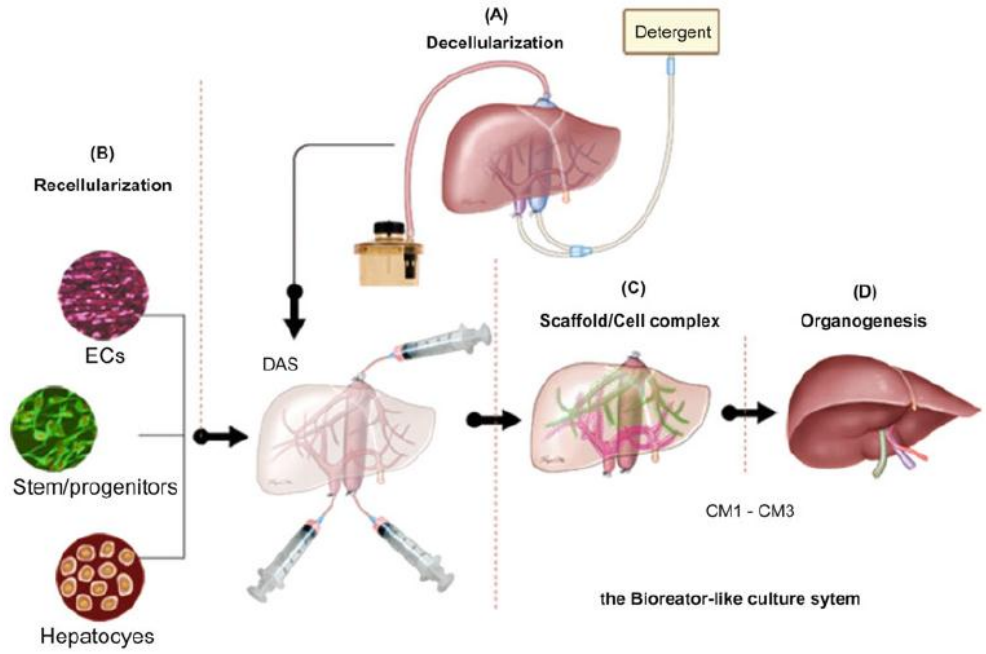

Figure 1: Strategy of liver organogenesis. (a) Generation of liver decellularized acellular scaffold (DAS) by detergent perfusion, with entry of fluid into the organ via portal vein cannulation. (b) Seeding of cells into the DAS. (c) Culture DAS–cell complex in the bioreactor-like culture system (BLCS). (d) Induction of liver organ formation with conditioned media (CMs) in the BLCS (from the paper of Yang *et al*[36])

SNAKE ECDYSIS-DERIVED SCAFFOLD FOR HEPATIC REGENERATION- AN IDEA BASED HYPOTHESIS

An idea regarding developing a novel scaffold, derived from snake Ecdysis which becomes an alternative functional material with sufficient mechanical strength for liver regenerative applications (Figure 2). Snake Ecdysis (Shedding of the skin), which is usually considered as snakes wastes, were acellularized, decalcified and fabricated into collagen scaffolds. The newly designed snake ecdysis scaffold for application in liver tissue engineering is attractive for the following reasons. Firstly, the acellular cells used in study differed from hydrogels due to biodegradable quality. Second, the derived acellular material is hydrophili, highly cyto-compatible with the host cells and with highly patterned structures that can readily promote cell conductive properties and bulk tissue integration for regenerating injured Hepatic tissues. Third, the derived acellular material is highly porous and well permeable to gas exchange. In summary, the present study has demonstrated the feasibility of the snake ecdysis –derived scaffold as a superior material for artificial liver regeneration.

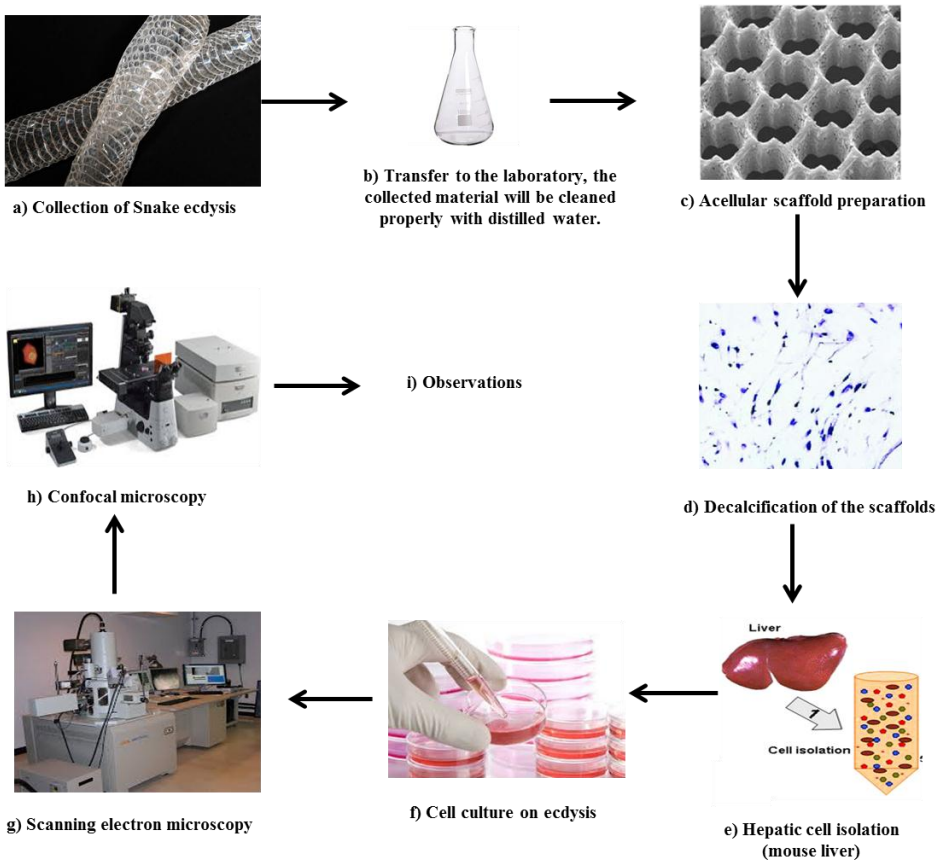

Figure 2: General hypothetical illustration of utility of snake ecdysis derived scaffolding on hepatic cells.

CONCLUSION

The synthesis make from the literature surveyed from various databases on ecdysis, tissue engineering, hepatic problems, scaffolds, organ regeneration etc. shown that this research idea can help to explain the functional requirements, and types, of materials used in developing state of the art of scaffolds and ecdysis and their implementation for applications in tissue engineering related to many health problems like hepatic problems. Furthermore, it describes the challenges and where future research and direction is required in this rapidly advancing field.

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